ORIGINAL PAPER

Christina E. Adamsen · Jens K. S. Møller ·
Ramadan Hismani · Leif H. Skibsted

Thermal and photochemical degradation of myoglobin pigments in relation to colour stability of sliced dry-cured Parma ham and sliced dry-cured ham produced with nitrite salt

Abstract Lipophilic and hydrophilic extracts of the red pigments from Parma ham and nitrosylated pigment of dry-cured ham produced with nitrite salt were prepared with acetone/water (75/25 v/v %) solution and aqueous phosphate buffer, respectively. The spectral characteristics differed for both the lipophilic and the hydrophilic Parma ham pigment compared with the dry-cured ham produced with nitrite salt. The red lipophilic pigment(s) extractable from Parma ham was(were) found to be very stable towards thermal degradation in acetone/water (75/25 v/v %) solution for temperatures up to 70 °C in contrast to the lipophilic pigment(s) extractable from dry-cured ham produced with nitrite salt, which was(were) found to have an energy of activation of 99 kJ/mol for thermal degradation. In contrast, quantum yields for photodegradation of the lipophilic ham pigments exposed to 366 nm (420 nm) monochromatic light were larger for Parma ham than for nitrite-cured ham [1.6×10^{-5} (6.9×10^{-6}) versus 1.6×10^{-6} (2×10^{-6}) mol einstein^{-1}] as determined for acetone/water (75/25 v/v %) solution. In agreement with these findings for the extracted lipophilic pigments, sliced Parma ham showed better colour stability than sliced dry-cured ham produced with nitrite salt, when stored in the dark at low oxygen concentration, in contrast to a faster initial discolouration for Parma ham when exposed to light, as shown for chilled storage for 35 days under retail conditions for the two products each packed at two oxygen levels (0.4 and 21%).

Keywords Dry-cured Parma ham · Dry-cured ham produced with nitrite salt · Colour stability · Photodegradation · Thermal degradation

C. E. Adamsen · J. K. S. Møller · R. Hismani · L. H. Skibsted (✉)
Food Chemistry, Department of Dairy and Food Science,
The Royal Veterinary and Agricultural University,
Rolighedsvej 30, 1958 Frederiksberg C, Denmark
e-mail: ls@kvl.dk
Tel.: +45-32283221
Fax: +45-35283344

Introduction

Colour is an important sensory property of many foods and contributes greatly to their overall acceptability. The characteristic pink colour of nitrite-cured meat products has a special role in consumer acceptance, as a stable pink colour is usually associated with high quality of such processed meat products [1]. Curing with salt added nitrite/nitrate is generally used to achieve a stable red colour of both dry-cured and brine-cured meat products. However, the unique red colour of dried cured hams from the Parma region in Italy develops from muscle myoglobin during maturation solely as a consequence of salting with sodium chloride and dehydration. Parma hams are made only from pork legs and sodium chloride in the form of sea salt from the Mediterranean Sea. The ham is renowned for its bright red colour and its characteristic flavour and texture [2, 3].

The pathway of formation and even the exact identity of the pigments of Parma ham still remain unknown. The pigment of Parma ham is, however, now known to be different from nitrosylmyoglobin, which is formed in nitrite-cured meat products, as has been shown by electron spin resonance spectroscopy [4, 5, 6]. Virgili et al. [7] have suggested that the low molecular weight compounds containing electron donor atoms (mainly nitrogen), which are formed during maturation of Parma ham, in particular peptides and amino acids resulting from an extensive proteolysis, may play a role as ligands in myoglobin.

Sakata et al. [4] showed that pigments extracted with water from Parma ham were stable in the pH region 5–10 under ambient aerobic conditions. Other studies dealing with the stability of Parma ham pigments have shown that pigments extracted with aqueous phosphate buffer from Parma ham are stable towards oxidation by ferricyanide and also rather stable towards autoxidation at ambient conditions [8]. The same study also showed that the red pigments extracted with acetone/water (75/25 v/v %) solution from Parma ham or with aqueous buffer are stable in darkness, but degraded within 3 days when kept

under light at 4 and 20 °C [8]. Furthermore, no effect of vitamin E on colour intensity or stability was observed in Parma ham manufactured from pigs supplemented with vitamin E in comparison with pigs from a normal feeding regime [9]. In the same study the colour stability of sliced Parma ham packed in vacuum or in a protective atmosphere (85% N_2 and 15% CO_2) exposed to fluorescent light was investigated, and it was concluded that the colour of the sliced Parma ham was maintained for about 2 months, as evaluated by sensory analysis [9].

The stable red pigment of Parma ham is also of interest from a more general meat processing point of view, since it presents future perspectives for manufacturing meat products with a desirable colour without the use of either nitrate or nitrite. Safety aspects of using especially nitrite as an additive have been discussed over the past decades.

The objective of the present study was to investigate the physicochemical properties of the Parma pigment as extracted from the ham in comparison with the pigments extracted from dry-cured ham produced with added nitrite. More specifically, the susceptibility of the pigments towards photodegradation and thermal decomposition were investigated for pigments isolated from Parma ham and from dry-cured ham produced with nitrite salt. Moreover, the light sensitivity of both of the meat products was evaluated in a chill storage experiment, where sliced Parma ham and sliced, dry-cured ham produced with nitrite salt packed in high and in low oxygen atmospheres were stored in the dark or exposed to light of 1,000 lx intensity under retail conditions.

Materials and methods

Chemicals

Acetone, sulphuric acid (95–97%), hydrogen peroxide (30%), sodium thiosulfate, calcium chloride dihydrate, phenantrolin, sodium acetate, nitric acid (65%), silver nitrate, vanadium(III) chloride and sodium nitrite were all obtained from Merck (Darmstadt, Germany). Chloroform and methanol were obtained from Lab-Scan (Dublin, Ireland.). 2′,7′-Dichlorofluorescein, N-(1-naphthyl)ethylenediamine dihydrochloride (NEDD), Griess reagent, and sulfanilamide (SULF) were purchased from Sigma–Aldrich (Steinheim, Germany). Potassium ferrioxalate was obtained from K & K Laboratories, Division of Biomedicals (Cleveland, OH, USA). All chemicals were of analytical grade and were used without further purification. Water was purified through a Millipore Q-Plus unit (Millipore, Bedford, MA., USA).

Meat samples

Fully matured Parma ham (total processing time of 18 months) was obtained from a local producer in Parma through Stazione Sperimentale per I'Industria delle conserve Alimentari, Parma, Italy. The dry-cured ham made with nitrite salt (assigned standard dry-cured ham hereafter) was obtained from Tulip International (Viby J., Denmark).

Packaging and storage conditions

Sliced ham (thickness 3 mm) samples were placed in 22.5 cm× 15.5 cm bags of a laminated packaging material (polyamide/ethyl vinyl alcohol/polyamide 40 μm/linear low-density polyethylene 75 μm, AMCOR, Unibags, Denmark) with an oxygen transmission rate of 0.45 cm^3 m^{-2} atm^{-1}24 h^{-1} (measured at 23 °C and 5/95% relative humidity), a carbon dioxide transmission rate of 1.8 cm^3 m^{-2} atm^{-1}24 h^{-1} (measured at 23 °C and 5/95% relative humidity) and a water-vapour transmission rate of 3.9 g cm^{-2} (measured at 23 °C and 50% relative humidity), which were heat-scaled. The volume ratio of headspace to meat was 12:1 and the oxygen concentration in headspace was 21% or 0.4%. The samples were stored in the dark or exposed to light in chill cabinets (Termaks series 6000, Termaks, Solheimsviken, Norway), and the temperature was monitored continuously during storage at two different locations in each chill cabinet using data loggers (Tinytalk II–Temp Loggers, RS Radio Parts, Copenhagen, Denmark). The mean storage temperature was 6.2 °C. Fluorescent tubes (Philips New Generation TLD, 36 W/830, Copenhagen, Denmark) were used for illumination, yielding an illuminance of about 1,000 lx at the surface of the meat samples. The samples stored in the dark were covered with black plastic. The starge period was 35 days.

Analysis for the storage experiment

On day 0, randomly selected samples (n=3) from Parma ham and from standard dry-cured ham were analysed for:

– Total fat (as grams of fat per 100 g of meat) by the method of Folch et al. [10].
– pH as measured in a 1:1 meat/water homogenate made from 5 g of meat and 5 ml water homogenized for 1 min at 15,000 rpm using a Ultra Turrax homogenizer (Janke & Kunkel, Kika Labortechnik, Staufen, Germany). The pH was measured using a pH meter (713 pH meter, Ω Metrohm, Herisau, Switzerland).
– Salt content (as grams of salt per 100 g of meat) determined as chloride using 1 g of homogenised meat sample accurately weighed and transferred into a 100 ml conical flask together with 50 ml boiling water. A 1 ml aliquot of 2.00 M nitric acid and five drops of 0.1% fluorescein indicator were added after boiling prior to titration with 0.10 M silver nitrate.
– Protein content determined as grams protein per 100 g of meat. Lean meat was trimmed for any visible fat, chopped very fine and approximately 2 g was weighed accurately on non-nitrogenous paper (AGF 2572 12×12 cm, Frisinette, Ebeltoft). The meat sample and the paper were transferred to a Kjeldahl tube and destroyed in a Tecator 2020 digester (Foss Tecator, Höganäs, Sweden) using a standard procedure prior to distillation in a Tecator 1026 distilling unit combined with a Titralab TIM 900 titration manager and an ABU 900 autoburette (Radiometer, Copenhagen, Denmark). A reagent blank was measured to estimate the content of the nitrogen in the reagents. The factor used to convert percentage N to percentage crude protein was 6.25.
– Nitrite content (ppm) determined by the Norwitz–Keliher method [11], in which the NEDD concentration was doubled as described by Binstok et al. [12].
– Nitrate content (ppm) determined by a method based on that of Miranda et al. [13] where vanadium(III) reduces nitrate to nitrite. The digestion, clarification and filtration of the meat samples were performed as described by Norwitz and Keliher [11]. The filtrate (25 ml) was mixed with 12.5 ml NEDD (1 w/v in H_2O), 12.5 ml SULF (2% w/v in 5% HCl), 25 ml of a saturated solution of VCl_3 in 1 M HCl and water to make 100 ml. The absorbance was measured at 542 nm and total nitrite content calculated using a sodium nitrite calibration curve. The nitrate content was calculated as the difference between the nitrite content before and after addition of vanadium(III).

– Water content (as grams of water per 100 g of meat) measured by a moisture analyser (Sartorius MA 30, Decagon Devices, Pullman, WA, USA).
– Water activity measured using a CX-2 water activity analyser (AQUA LAB, Pullman, WA, USA).

The gas composition of the package headspace was measured using a CheckMate 9900 gas analyser (PBI Dansensor, Ringsted, Denmark) and expressed as percentage O_2 and percentage CO_2.

The colour of the meat surface was measured every day for the first 4 days and later in the storage periods at appropriate intervals through the package film with a Color-guide 45/0 (BYK-Gardner, Geretsried, Germany) using the L^*, a^*, b^* coordinates (CIE $L^*a^*b^*$ colour system). A red colour was expressed as the a^* value, which increases with redness. The measurement was repeated on five randomly selected locations on each package for five packages for each type.

Pigment extraction

Pigments from the Parma ham or from the standard dry-cured ham were extracted with acetone/water (75/25 v/v %) solution and with aqueous phosphate buffer [6]. Extracts were kept in the dark and on ice and were used for measurements within 4 h. The concentration of the pigment extracted from Parma ham and standard dry-cured ham was calculated from spectrophotometric measurements: $\varepsilon = 199{,}200$ $M^{-1}cm^{-1}$ at 416 nm for acetone/water (75/25 v/v %) solution; $\varepsilon = 152{,}100$ $M^{-1}cm^{-1}$ at 424 nm for aqueous phosphate buffer (pH 6.00) for Parma ham [6]; $\varepsilon = 146{,}800$ $M^{-1}cm^{-1}$ at 421 nm for both acetone/water (75/25 v/v %) solution and aqueous phosphate buffer (pH 6.00) for standard dry-cured ham [14].

Photodegradation of ham pigments

A continuous-wave photolysis technique was employed during which a four-window cuvette with the ham extracts was exposed for a period of 12 h to monochromatic light (366 and 420 nm) selected by a monochromator from a Xe lamp. The light was focused by an optical system with a light condenser and shutter connected to a timer into the cuvette in the thermostated (25±0.5 °C) cell holder with magnetic stirring in a HP 8453 UV–vis diode-array spectrophotometer (Hewlettt-Packard, Palo Alto, CA, USA), and absorption spectra (from 300 to 700 nm at 2 nm intervals) were recorded at appropriate time intervals. Light intensities at 366 and 420 nm were determined by ferrioxalate actinometry [15]. Quantum yields for photooxidation of ham pigments extracted with acetone/water (75/25 v/v %) solution were determined from absorption spectra recorded during photolysis and light intensities as determined by actinometry [16]. The quantum yield defined as

$$\phi_{irr} = \frac{\text{moles of pigment reacted}}{\text{moles of photons absorbed by pigment}}$$

was calculated from the change in concentration of the pigment as monitored spectrophotometrically. The intensity of light at the wavelength of irradiation as determined by actiniometry was corrected to the fraction of light absorbed (A_{irr}) in the cuvette by the factor $1 - 10^{-A_{irr}}$ derived from the Lambert–Beer law.

Thermal degradation of ham pigments

Ham pigment (100 ml) extracted with acetone/water (75/25 v/v %) solution or aqueous phosphate buffer was kept at 30, 40, 50, 60 or 70 °C for 3 h in a thermostat. The spectra from 350 to 700 nm were recorded at 30 min intervals for 3 h with a Cintra 40 UV–vis spectrophotometer (VEB Analytical, GBC Scientific Equipment, Australia), except for 70 °C for which temperature spectra were measured at 15 min intervals. Extracts becoming turbid were centrifuged (5 °C, 15,000 rpm for 5 min) prior to the spectrophotometric measurements. The pigment concentration was followed from changes in absorbance at the Soret band [Parma ham pigment, acetone/water (75/25 v/v %) solution 416 nm; Parma ham pigment aqueous phosphate buffer 424 nm; standard dry-cured ham pigment, acetone/water (75/25 v/v %) solution 400 nm; standard dry-cured ham pigment, aqueous phosphate buffer 412 nm]. The decomposition of the extracted pigments was expressed as percent decomposed assuming that absorbance would decrease to zero for complete degradation:

$$\text{Relative pigment content}(\%) = \left[1 - \left(\frac{A_{t=0} - A_{t=x}}{A_{t=0}}\right)\right] \times 100\%.$$

Data analysis

Average values of fat, moisture, protein, NaCl, a_w, pH, nitrite and nitrate contents of Parma ham and standard dry-cured hams and the quantum yield for photodegradation were compared by the Student t-test in the Analyst application within the SAS system statistical software release 8.02 (SAS Institute, Cary, NC, USA). The spectral changes during thermal decomposition of pigment extracted from Parma ham and standard dry-cured ham with acetone/water (75/25 v/v %) solution and aqueous phosphate buffer were analysed using a kinetic model with a single-exponential decay corresponding to a first-order reaction. The analyses were carried out using Microcal Origin version 6.0 (Microcal Software, MA, USA). The first-order rate constants were further analysed according to the Arrhenius equation in order to estimate the activation energy (E_a) for thermal decomposition of the four pigments.

Results and discussion

Parma ham produced according to the official regulation in the Parma region in Italy and a standard dry-cured ham produced by the Danish meat industry and produced with salt-added nitrite were compared with respect to colour stability and pigment properties. The compositions of the two hams used for this comparison are presented in Table 1. The hams are rather similar in composition except for the content of nitrite and nitrate, which were both below the detection limit for the Parma ham. The salt content is significant higher and the water activity correspondingly lower for the standard dry-cured ham than for Parma ham.

Table 1 Approximate composition of the Parma ham and the standard dry-cured ham. Means with the same superscript letter in the same column are not significantly different at a 5% level

	Fat (g/100 g meat)	Moisture (g/100 g meat)	Protein (g/100 g meat)	NaCl (g/100 g meat)	a_w	pH	Nitrite (ppm $NaNO_2$)	Nitrate (ppm $NaNO_3$)
Dry-cured ham	6.2±1.9[a]	43.2±1.7[a]	35.7±1.0[a]	8.2±0.4[a]	0.879±0.004[a]	5.5±0.2[a]	10.2±1.5	4.8±0.4
Parma ham	6.9±0.6[a]	45.8±2.1[a]	34.9±2.8[a]	5.7±0.2[b]	0.913±0.002[b]	5.70±0.01[a]	<1	<1

Fig. 1a–d Redness parameter (a^* values) of sliced Parma ham and nitrite-based dry-cured ham during 35 days storage at 5 °C. **a** Sliced Parma ham packed with 21% headspace oxygen and an illuminance level of 1,000 lx (*squares*) or of 0 lx (*circles*). **b** Sliced Parma ham packed with 0.4% headspace oxygen and an illuminance level of 1,000 lx (*squares*) or of 0 lx (*circles*). **c** Sliced standard dry-cured ham packed with 21% headspace oxygen and an illuminance level of 1,000 lx (*diamonds*) or of 0 lx (*triangles*). **d** Sliced nitrite-based dry-cured ham packed with 0.4% headspace oxygen and an illuminance of 1,000 lx (*diamonds*) or of 0 lx (*triangles*)
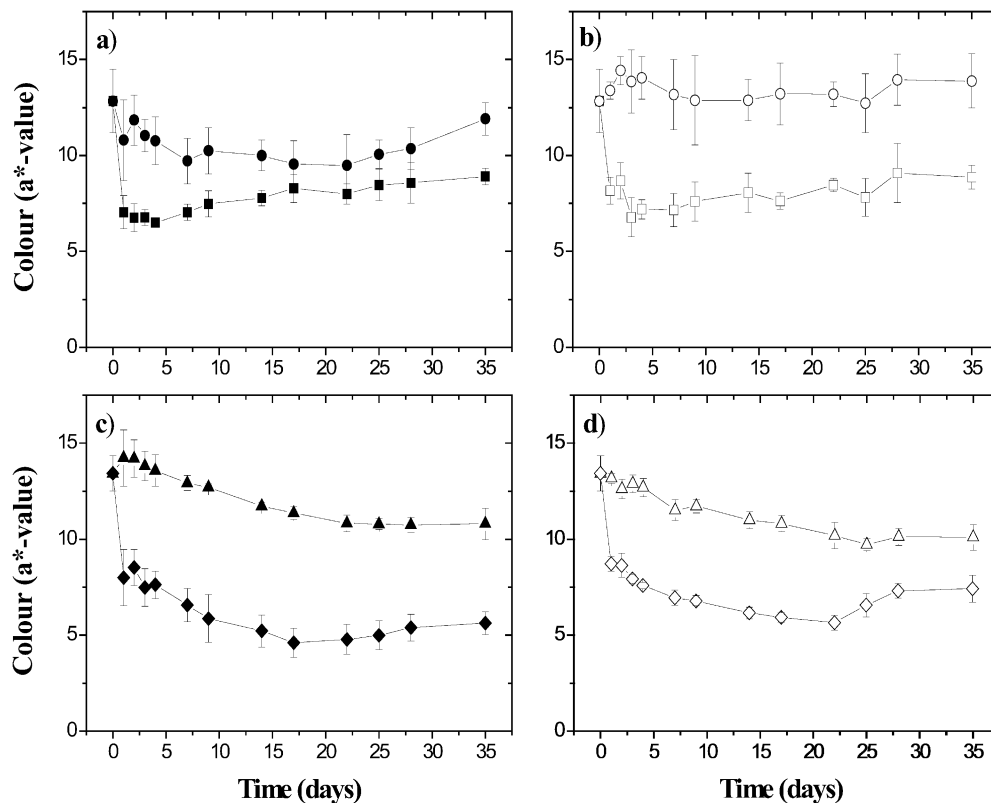
Colour stability during storage

The colour stability for sliced Parma ham and sliced standard dry-cured ham was monitored as the a^* value through a 35 day storage period in a chill cabinet under retail display condition. The change in the a^* value (redness) of Parma ham and standard dry-cured ham for two levels of oxygen in the headspace (21 and 0.4%) and two levels of illumination (0 and 1,000 lx) is shown in Fig. 1. The rates of discoloration for Parma ham and standard dry-cured ham exposed to light either in 21% or in 0.4% oxygen in headspaces were very similar, with both types of ham showing a significant drop in the a^* value from day 0 to day 1, and the rate of discoloration were surprisingly independent of the oxygen level. From Fig. 1a a slower rate of discoloration is evident for Parma ham stored in the dark in 21% oxygen and for standard dry-cured ham stored in the dark in 21% (Fig. 1c) or 0.4% (Fig. 1d) oxygen compared with the illuminated samples. However, for both types of ham stored in the dark under these conditions a significant discoloration still occurs, in contrast to what is seen for Parma ham stored in 0.4% oxygen in the dark (Fig. 1b), which maintains the red colour throughout the storage period. Illumination decreases colour stability for standard dry-cured hams and any difference between the rate of discoloration for standard dry-cured ham stored in 21% oxygen and in 0.4% oxygen was insignificant, when the product was exposed to light and also when it was kept in the dark, although a clear difference is seen between light-exposed

and light-protected samples. In contrast, the colour of Parma ham was found to be stable when the product was kept in the dark in an initial atmosphere of 0.4% oxygen.

During the storage period the gas composition of the package headspace was measured. For Parma ham and standard dry-cured ham stored in an initial 21% oxygen atmosphere in the headspace, a drop in oxygen content from 21% to below 5% was observed from the start of the storage to 20 days of storage. This decrease in headspace oxygen was observed both for samples exposed to light or for samples protected against light. For Parma ham and standard dry-cured ham stored in an initial atmosphere of 0.4% oxygen, an increase in oxygen content from 0.4% to approximately 1% was seen for 7 days of storage followed by a decrease in oxygen to below 0.2%. This sequence, which reflects the balance between oxygen consumption of the product and oxygen penetration though the packaging film, was observed both for samples stored exposed to light and for samples protected against light.

Spectral characterisation

The pigments in Parma ham and standard dry-cured ham were extractable with acetone/water solutions as has been shown previously [6, 17]. However, the extraction was not completed since the meat after extraction still displayed a weak pink colour. The absorption spectra in the visible region (350–650 nm) of Parma ham and
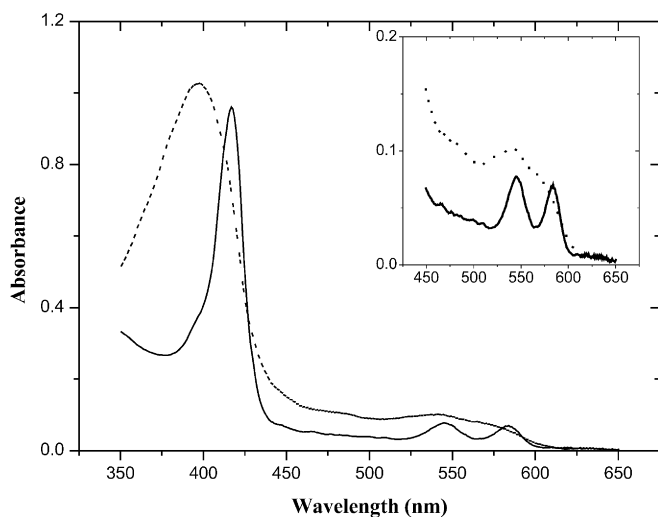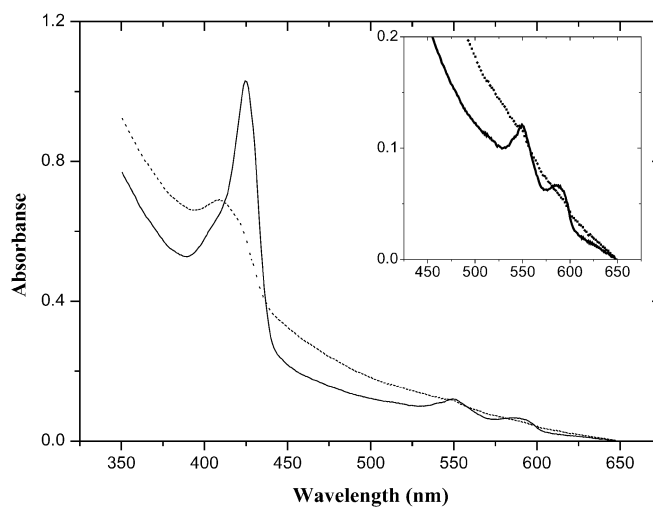
Fig. 2 Absorption spectra in the visible region of Parma ham pigment (*full line*) and standard dry-cured ham pigments (*dotted line*) extracted with acetone/water (75/25 v/v %) solution. In each case 10 g of ham was extracted with 90 g of extraction solvent. The *inset* shows the absorption region from 450 to 650 nm in detail
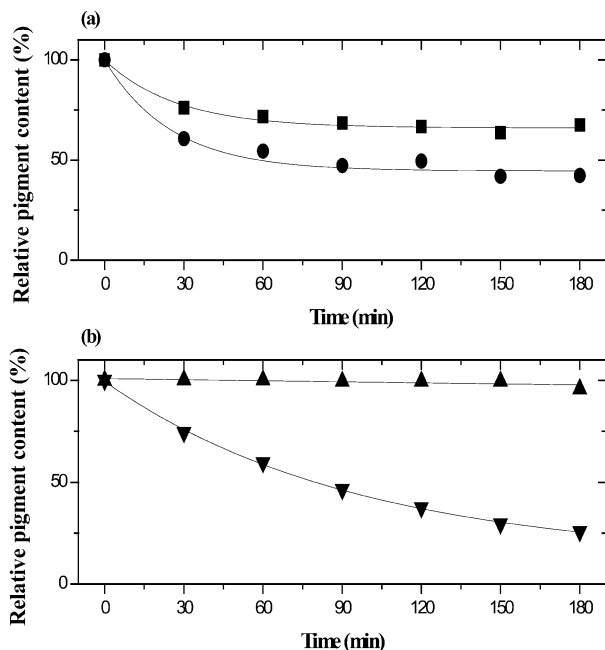

Fig. 3 Absorption spectra in the visible region of Parma ham pigment (*full line*) and standard dry-cured ham pigment (*dotted line*) extracted with aqueous phosphate buffer. In each case 10 g of ham was extracted with 90 g of extraction solvent. The *inset* shows the absorption region from 450 to 650 nm in detail

standard dry-cured ham pigments extracted with acetone/water solution were clearly different (Fig. 2). The Parma ham showed maxima at 418, 546 and 582 nm, in agreement with the findings of Møller et al. [6] and Morita et al. [17]. The standard dry-cured ham showed maxima at 396 and 540 nm, and shoulders at 475 and 570 nm as is typical for nitrosylated myoglobin derivatives [18]. The pigments from Parma ham were also extractable with aqueous phosphate buffer and as for acetone/water extraction, the extracted meat had a weak pink colour. The pigments of standard dry-cured ham were poorly extracted by aqueous phosphate buffer and the extracted meat retained a pink colour. The absorption spectra (350–650 nm) of pigment extracted with aqueous phosphate buffer from Parma ham and standard dry-cured ham were mutually different and each was different from the spectrum for the acetone/water extract (Fig. 3). The aqueous extract of Parma ham showed maxima at 424, 550 and 586 nm, all redshifted relative to the acetone/water extract, while the standard dry-cured ham showed one clear maximum in the Soret band at 396 nm.

Photodegradation

The quantum yield for photodegradation of the pigments extracted with acetone/water solution was determined at 25 °C, at which temperature thermal degradation is minimal (see later). The quantum yield for photodegradation provides an objective measure of the light sensitivity of Parma ham pigment and pigment from standard dry-cured ham at irradiation wavelengths of 366 and 420 nm, which were selected as characteristic for fluorescent light (366 nm) and for an intense absorption for the pigment (420 nm), respectively. From Table 2, it is seen that the quantum yield for photodegradation was found to depend both on the pigment type and on the wavelength of irradiation. The pigment extracted by acetone/water solution from Parma ham is more sensitive to light than the pigment extracted from standard dry-cured ham. The quantum yield for photodegradation of standard dry-cured ham pigments exhibits at most a moderate dependence on the wavelength, in contrast to the Parma ham pigments, which are more labile for illumination at the lower wavelengths of irradiation (366 nm).

Table 2 Quantum yields (ϕ) for photodegradation of pigments extracted from Parma ham and standard dry-cured ham for exposure to monochromatic light at 25 °C (420 and 366 nm). Pigments were extracted with acetone/water (75/25 v/v %) solution, see Fig. 2. Means with the same superscript letter in the same column are not significantly different at a 5% level. Means with the same underlined italized letter in the same row are not significantly different at a 5% level

	Irradiation at 420 nm	Irradiation at 366 nm
	ϕ_{420} (mol einstein^{-1})	ϕ_{366} (mol einstein^{-1})
Parma ham pigment	$6.9 \pm 2.8 \times 10^{-6}$ ($n=3$)[a,*a*]	$1.6 \pm 0.2 \times 10^{-5}$ ($n=2$)[a,*b*]
Standard dry-cured ham pigment	$2.0 \pm 1.1 \times 10^{-6}$ ($n=2$)[b,*a*]	$1.6 \pm 0.1 \times 10^{-6}$ ($n=2$)[b,*a*]

Fig. 4 Thermal degradation at 60 °C of pigments extracted from **a** Parma ham (*squares*) and standard dry-cured ham extracted with aqueous phosphate buffer (*circles*) and of **b** Parma ham (*up triangles*) and standard dry-cured ham extracted with acetone/water (75/25 v/v %) solution (*down triangles*) followed spectrophoto-metrically at the absorption maximum in the Soret band and expressed in relative concentration. The decrease in pigment concentration were analysed using a kinetic model with a single-exponential decay corresponding to a first-order reaction (*full lines*)

Thermal degradation

The thermal degradation of pigments extracted from Parma ham and standard dry-cured ham with acetone/water solution and aqueous phosphate buffer was inves-tigated at 30, 40, 50, 60 and 70 °C. For Parma ham pigment extracted with acetone/water solution, no thermal degradation was observed in the temperature interval investigated. Thermal degradation was observed for the three other types of pigments, and the rate increased with increasing temperature. The relative pigment concentra-tion is shown in Fig. 4 as function of time for pigments extracted with acetone/water solution and for aqueous phosphate buffer at 60 °C for both types of ham. The spectral changes during thermal decomposition of the pigments were analysed using a kinetic model with a single-exponential decay,

$$\text{Relative concentration} = 100 \times e^{-k_{obs}t}$$

corresponding to a first-order reaction as shown in Fig. 4. An adequate description of the degradation of the pigments seems to be obtained by first-order kinetics, as seen most clearly for the standard dry-cured ham pigment extracted with acetone/water solution (Fig. 4b). The temperature dependency of the observed first-order rate constant was analysed according to the Arrhenius equa-tion for the pigment extracted from standard dry-cured
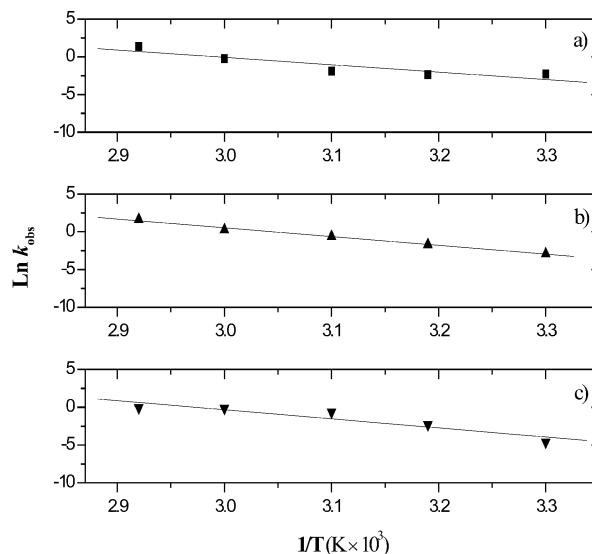
Fig. 5a–c The natural logarithms of the initial first-order rate constants (ln k_{obs}) as a function of the reciprocal absolute temperature ($T^{-1} \times 10^3$). The plots are Arrhenius linearised equation fits and the slop is $-E_a/R$. **a** Parma ham extracted with aqueous phosphate buffer (*squares*), **b** standard dry-cured ham pigment extracted with aqueous phosphate buffer (*up triangles*), and **c** standard dry-cured ham extracted with acetone/water (75/25 v/v %) solution (*down triangles*)

ham with acetone/water solution and with aqueous phosphate buffer and for Parma ham extracts made with aqueous phosphate buffer, see Fig. 5. Activation energies were estimated and E_a had the value 81 kJ mol^{-1} for the Parma pigment extracted with aqueous phosphate buffer, while it was 99 and 96 kJ mol^{-1} for the pigment extracted from standard dry-cured ham with acetone/water solution and aqueous phosphate buffer, respectively. Notably, the lipophilic Parma ham pigment did not degrade even at 70 °C.

The physicochemical properties of Parma ham extracts prepared with acetone/water solution and aqueous phos-phate buffer differed for those of standard dry-cured ham both in extractability and in spectral characteristics; however, the spectral characteristics were all in agree-ment with some form of a modified myoglobin with an intense Soret band around 400 nm. It is known that the maturation process of Parma ham increases the fraction of a lipophilic myoglobin derivative corresponding to the pigment(s) extracted by acetone/water solution [6]. This pigment, which dominates in the fully matured Parma ham, is the most stable towards thermal degradation, when the four different pigments are compared, in agreement with the high colour stability of unsliced Parma ham (Fig. 4).

No difference in colour stability could be observed for Parma ham and standard dry-cured ham stored in 21% or 0.4% oxygen in the package headspace, when samples were exposed to light. This part of the investigation further showed that the colour fades when sliced Parma ham is illuminated in retail packages containing even low

headspace oxygen (0.4%). In order to preserve the colour of Parma ham from a practical point of view, it is accordingly necessary to reduce residual oxygen in the headspace of packages, use packaging material with a high oxygen barrier, and apply a package lid that blocks light.

From the values of the quantum yield determined for photodegradation of the lipophilic Parma ham pigment and the pigment from standard dry-cured ham, it can be concluded that Parma ham pigment is more light-sensitive than standard dry-cured ham pigment extracted with acetone/water solution for illumination at both 366 and 420 nm. However, the quantum yields were found to be rather small, and the differences seen are not reflected in a real difference in the colour stability of Parma ham and standard dry-cured ham exposed to light under retail condition (Fig. 1). However, it is clear from the storage experiment with the two types of hams, that Parma ham shows a faster initial discolouration, in agreement with the larger quantum yields, but the total discolouration is less significant especially for high oxygen storage. The quantum yields for nitrosylmyoglobin as determined in model systems show the same moderate wavelength dependency as found especially for the pigment isolated from the nitrite-cured ham [16, 19].

The thermal degradation of isolated pigment clearly demonstrated that the pigments are sensitive to heat. The energies of activation are relatively high as was also found for nitrosylmyoglobin in aqueous solution [16]. It is notable that these high energies of activation for degradation of the pigments are in agreement with the high colour stability seen for dark storages of especially Parma ham (Fig. 1). The lipophilic Parma ham pigment showed no sign of degradation in the temperature interval 30–70 °C.

In summary, the experiments clearly demonstrate that Parma ham pigments differ from the nitrosylated pigments in standard dry-cured ham produced with nitrite salt, with respect to a number of properties. Most interestingly, the lipophilic Parma ham pigment extracted with acetone/water solution exhibits superior stability towards thermal degradation and can be considered to be stable even at room temperature as long as light is excluded.

A more detailed understanding of the chemical processes behind degradation of the Parma ham pigments will, however, have to await results from our current work, which is concentrated on identifying the exact chemical structure of the Parma ham pigment(s). However, it seems evident, that the chemical properties of the lipophilic pigments of the two types of ham predict better the colour stability of the actual sliced product than pigments extracted with aqueous buffer.

References

1. Fox JB Jr (1966) J Agric Food Chem 14:207–210
2. Parolari G (1996) Food Sci Technol Int 2:69–78
3. Sakata R (2000) Anim Sci J 71:1–16
4. Sakata R, Niu J, Morita H, Nagata Y (1998) In: Diestre A, Monfort JM (eds) Congress proceedings, meat consumption and culture. 44th international congress of meat science and technology, 30 August–4 September 1998, Barcelona, Spain. Estrategias Alimentarias, Madrid, Spain, pp 988–989
5. Morita H, Niu J, Sakata R, Nagata Y (1996) J Food Sci 61:1021–1023
6. Møller JKS, Adamsen CE, Skibsted LH (2003) Eur Food Res Technol 216:290–296
7. Virgili R, Parolari G, Bordini CS, Schivazappa C, Cornet M, Monin G (1999) J Muscle Foods 10:119–130
8. Sakata R, Morita H, Norimatsu T, Niu J (1999) Jpn J Swine Sci 36:124–129
9. Zanardi E, Novelli E, Ghiretti GP, Dorigoni V, Chizzolini R (1999) Food Chem 67:163–171
10. Folch J, Lees M, Stanley GHS (1957) J Biol Chem 226:497–509
11. Norwitz G, Keliher PN (1987) Analyst 112:903–907
12. Binstok G, Campos CA, Gerschenson LN (1996) Meat Sci 42:401–405
13. Miranda KM, Espey MG, Wink DA (2001) Nitric Oxide Biol Chem 5:62–71
14. Millar SJ, Moss BW, Stevenson MH (1996) Meat Sci 42:277–288
15. Hatchard CG, Parker CA (1956) Proc R Soc Lond Ser A 235:518–536
16. Andersen HJ, Skibsted LH (1992) J Agric Food Chem 40:1741–1750
17. Morita H, Sakata R, Sonoki S, Nagata Y (1994) Anim Sci Technol 65:1026–1033
18. Hornsey HC (1956) J Sci Food Agric 7:534–540
19. Møller JKS, Bertelsen G, Skibsted LH (2002) Meat Sci 60:421–425